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CARBOXYLESTERASES OF THE TESTES: ROLE IN ACTIVATION OF TOXICANTS

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13. ABSTRACT (Maximum 200 words)

Organ specific distribution of carboxylesterases (Western blotting) was determined to be liver] lung = testes = fat] pancreas] kidney. Carboxylesterase distribution among cell types of the testes was examined by in situ hybridization techniques. Results were inconclusive, as both the probe and the control hybridized to tissues macromolecules. More refinement of this techniques should provide better results. Other accomplishments include examination of the down-regulation of carboxylesterase levels by glucocorticoids. Apparently esterase levels are most dramatically down-regulated (approximately 6-fold) by dexamethasone phosphate (60 mg/kg x 5 days, i.p.) in the testes compared to the other tissues containing this enzyme.

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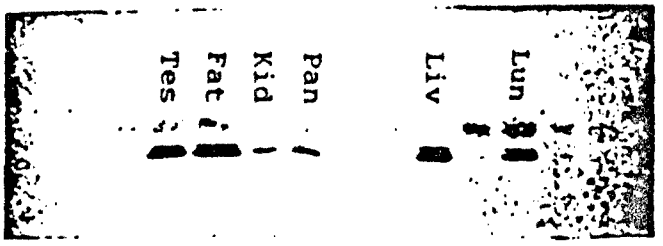
ACT
89-0190

PROGRESS REPORT
8-1-89 TO 7-31-90
Air Force Office of Scientific Research
New Investigator Award
"Carboxylesterases of the Testes: Role in Activation of Toxicants"

Progress towards proposed Specific Aims:

1. Organ specific distribution of the carboxylesterases was determined by Western blotting using anti-carboxylesterase antibodies and homogenates (20 ug total protein) of the various tissues. Relative abundance of the carboxylesterases was determined to be liver > lung = testes = fat > pancreas > kidney (see Fig. 1). At least 2 distinct bands were visualized in all of these tissues except the kidney, which is suggestive of multiple carboxylesterases forms, all of which are immunoreactive with the polyclonal antibodies.



2. Carboxylesterase distribution among cell types of the testes was examined by in situ hybridization techniques. Tissue slices were prepared as paraformaldehyde or immunobed sections. Samples were pre-hybridized and then hybridized with a ^{35}P-labeled antisense RNA or a sense RNA (control) made from a carboxylesterase clone inserted into the vector pGEM, in order to detect carboxylesterase-related sequences. Initial results were inconclusive, as both the probe and the control hybridized to tissue macromolecules (see Fig. 2). More refinement of this technique (to establish increased stringency without releasing the tissue section from the glass slide) should provide better results.

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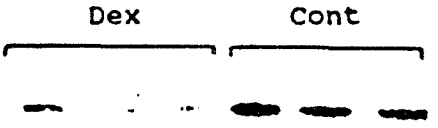
3. Establishment of the co-cultures of testicular cell types was deferred, due to down time while the lab at University of Maryland was being set up and staffed. This method will be re-established in Dr. Robert Chapin's laboratory at NIEHS and the questions with DEHP and TOCP will be addressed collaboratively. These studies are pending resolution of the carboxylesterase localization studies, described in 2. above.

4. Other accomplishments related to the specific aims proposed in this grant include examination of the down-regulation of carboxylesterase levels by glucocorticoids (funded by the PMAF grant, identified below). Apparently esterase levels are most dramatically down-regulated (approximately 6-fold) by dexamethasone phosphate (60 mg/kg x 5 days, i.p.) in the testes (see Fig. 3) compared to the other tissues containing this enzyme.



Personnel:

Staffing for this project included Ms. Maria Calabrese (undergraduate student, part-time 1 day per week for the year), Mr. Jian-Ming Mei (graduate student, part-time for 6 weeks), and Mr. Tom Cooney (prospective graduate student, part-time for 4 weeks). No full-time staff was available during the first project year.

Budget Report:

The purchases of equipment and supplies to furnish the laboratory were in accordance with the modified budget submitted to the Society of Toxicology Office and approved by Joan Cassedy and Jan Cervany (copy attached). The final budget report will be available after the conclusion of the grant period, and can be obtained from Michael Gentry, Dept. of Pharmacology and Toxicology, School of Pharmacy, 20 N. Pine St., University of Maryland, Baltimore MD 21201, (301) 328-2976.

Manuscripts and Abstracts:

1. "Comparisons Between Rat and Human Liver Carboxylesterases", presentation at the 1990 IUPHAR Meeting, Amsterdam, The Netherlands.

2. "Human Liver Carboxylesterase: cDNA Cloning and Sequencing", presentation at the 1990 FASEB Meeting, Washington, DC.

3. "Human Liver Carboxylesterase: cDNA Cloning and Sequencing and Evidence for a Multigene Family", manuscript in preparation for submission to FEBS Letters.

Other Support:

"Distribution and Regulation of Carboxylesterases", Starter Grant from the Pharmaceutical Manufacturers Association Foundation, $10,000/1 year (starting 1-1-90).

Extensions:

This project will not be continued after the conclusion of the grant period. The new address for the principal investigator will be (effective 8-1-90):

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